Accordingly, in the present invention there are provided nucleic acid constructs (claims 1, 2, 4-6 and 8-10) and embryonic stem (ES) cells containing such nucleic acid constructs (claims 12-16 and 18-26). The invention nucleic acid constructs and ES cells containing such constructs are used in methods for preparation of transgenic embryonic stem cells and transgenic animals. In those embodiments wherein the ES cells contain a germ-line specific promoter operatively linked to the recombinase encoding gene, the transcriptionally active selectable marker can be excised by passage of the genome derived from said embryonic stem cells through gametogenesis. ES cells obtained by crossing the genome of the transgenic gamete with a wild type genome can be used to obtain ES cells in which the transgene is stably incorporated into the genome, but the selectable marker can be is excised. Excision of the marker without excision of the allele of interest allows an observed phenotype to be ascribed confidently to the mutation of interest rather than to some combination of that mutation and the transcriptionally active marker. For the creation of tissue-specific or inducible mutations, site-specific recombinases can be used in an initial step to excise the marker, and, in combination with a tissue-specific or inducible recombinase transgene, to subsequently excise some essential component of the target of interest in the intact animal.

Claims 1-45 were pending before this response. Claims 1-10, 12-16, 18-26, and 28-45 remain pending, with claims 11, 17 and 27 being canceled herein, and with claims 1, 2, 4-6, 8-10, 12-26 and 28-44 presently under prosecution. Claims 10, 13, 15, 25, 26, 28, 31, 32, 35, 36, 39 and 40-44 are amended herein to further clarify the meaning and scope of the invention. These amendments add no new matter. Claims 3, 7 and 45 have been withdrawn from consideration as drawn to a presently non-elected invention pursuant to a restriction requirement asserted by the Examiner. The claims withdrawn from prosecution will be maintained in the Application pending final disposition of the elected claims.

Reduction in Length of the Abstract

The Office Action alleges that the Applicants have not complied with MPEP § 608.01(b) by using proper language and format for an Abstract of the disclosure because the Abstract of the disclosure exceeds 250 words. To reduce the number of words in the Abstract, Applicants submit herewith an amendment that limits the number of words therein to less than 250. Applicants submit that the amended Abstract is in accordance with MPEP § 608.01(b).

The Restriction Requirement

Applicants respectfully traverse the requirement for restriction of the claims under 35 USC § 121 in the Office Action, which proposes to divide the claims into two groups as follows:

> Group I (claims 1, 2, 4-16 and 18-44) drawn to methods of making transgenic animals classified in class 800, subclass 21 and
>
> Group II (claims 1, 3-8, 10-11, 17-22, 24-27 and 45) drawn to methods of making transgenic plants, classified in class 800, subclass 278.

Applicants disagree with the Examiner's assertion that the inventions of Groups I and II are distinct and have acquired a separate status in the art as shown by their different classification, such that the search required for Group I is not required for Group II (Office Action, page 3).

Applicants respectfully submit that the Group I and Group II claims include overlapping subject matter. For instance, the nucleic acid constructs of claims 1 and 4-8 can be used to obtain embryonic stem cells of claims 18-22 that are useful for obtaining either transgenic plants or animals, and the method of conditional assembly of functional genes of claim 43 is also useful in the production of either transgenic plants or animals. Applicants submit that the Examiner's consideration of the patentability of the nucleic acid constructs and embryonic stem cells of Group I will therefore, necessarily include a search of the art relating to the patentability of the

nucleic acid constructs and embryonic stem cells containing such nucleic acid constructs of Group II. Therefore, to maximize convenience and expediency for both the Examiner and the Applicants, the patentability of the nucleic acid constructs and of the embryonic stem cells containing them of Group II should be considered along with the patentability of those of Group I.

In view of the above remarks, reconsideration of the requirement for restriction is respectfully requested.

A provisional election to prosecute the invention of Group I claims was made with traverse during a telephone conversation with Stephen E. Reiter on November 23, 1998. In order to be fully responsive, Applicants hereby confirm the election with traverse of the Group I claims (i.e., claims 1, 2, 4-16 and 18-44) for prosecution on the merits. The non-elected claims are retained in the application pending final disposition of the elected claims.

The Rejection Under 35 USC § 112, First Paragraph

Applicants respectfully traverse the rejection of claims (the Office Action does not indicate which claims are rejected) for alleged indefiniteness under 35 U.S.C. § 112, First Paragraph, on the grounds that the Specification allegedly does not provide enablement for the full scope of the claims. Since the Examiner admits that the specification is enabling for certain specific embodiments illustrated in mice in the Examples of the Specification (Office Action, Paragraph 9, lines 2-19), Applicants submit that the question at issue is not lack of enablement *per se*, but the scope of enablement.

First, Applicants respectfully disagree with the characterization in the Office Action of the subject matter admitted by the Examiner to be enabled because it contains at least one prominent misrepresentation in the following description:

> ...a method of making ES cells wherein recombination of a second marker gene occurs comprising obtaining a male mouse whose genome comprises a nucleic acid construct comprising a mouse protamine 1 promoter operatively linked to Cre recombinase wherein expression of Cre recombinase occurs in the sperm, heart and spleen, breeding said male mouse to a female mouse whose genome comprises a marker gene construct comprising a first marker gene flanked by lox P target sites wherein said first marker gene is inserted between the promoter and the coding region of a second marker gene that is not expressed, [and] obtaining second generation ES cells wherein recombination of a second marker gene construct occurs...

(Office Action, pages 4 and 5, emphasis added). However, Applicants have shown that expression of Cre recombinase in the heart and spleen tissue is *de minimus* in all strains of mice tested. The Specification teaches that the signal in the PCR assay of the mouse strain having the highest expression in heart was "approximately equivalent to that expected if the ratio between recombined and unrecombined alleles was 1:104" (Specification page 17, lines 28-30). Expression in spleen was even lower. Regarding the efficiency of the germline specific expression of recombinase in the invention methods, the Specification teaches that recombinase nucleic acid constructs are "expressed at high levels in the germ line but not to a functionally significant extent in either ES cells or embryonic or adult somatic tissues" (Specification, page 15, lines 18-25, emphasis added). Applicants respectfully submit that a 100-fold or greater expression of the recombinase gene in testes than in the heart, brain and spleen adequately shows that the germ-line promoter operatively linked to the recombinase gene functioned as predicted. The Examiner has provided no reasons in support of the conclusion that expression of *de minimus* amounts of the recombinase in other tissues of the body would occur every time, or that, if it did occur, would be detrimental, i.e., "functionally significant." Those of skill in the art know that in healthy wild type individuals a recombinase is expressed in many different tissue types. Thus, Applicants respectfully submit that the Examiner mischaracterizes the scope of enablement provided in the Specification by representing that the expression of Cre recombinase under the control of a germ line specific promoter is so non-specific as to constitute the non-enablement of the present claims.

In addition, Applicants respectfully submit that the rejection for lack of enablement as to the scope of the invention contains further assertions that mischaracterize the enablement of the invention provided in the Specification. For example, the Examiner asserts:

> The specification does not enable the use of the nucleic acids or the embryonic stem (ES) cells as claimed because the specification does not demonstrate functional expression of any gene, does not correlate the expression of a marker gene to functional expression of any gene, provide a use for ES cells or mice expressing a non-functional marker gene or correlate ES cells or mice expressing a functional marker gene to ES cells or mice expressing other genes that may be of use. . . . The production of a ProCre/P2Bc mouse alone is not of use because . . . expression of a marker gene is not of apparent use as disclosed in the specification.

(Office Action, pages 8-9). Applicants respectfully submit that the purpose of the Examples in the Specification is merely to illustrate the invention and the claims are not intended to be limited to the scope of the Examples. Further, Applicants submit that the above statement, particularly the phrase ("not of use"), pertains to lack of utility, which is properly raised under 35 U.S.C. §101, rather than under 35 U.S.C. §112, Second Paragraph.

In fact, far from being "not of use," the creation of transgenic animals according to claims 32, 35, 40 and 44 and Applicants' genetically modified ES cells useful for obtaining such transgenic animals which contain a mutation of interest but in which the selectable marker has been eliminated, have a distinct utility described in the Specification, which teaches:

> ...alleles containing a single recombinase target site and a mutation of interest can be produced in the progeny of ES cell chimeras without any investment of time, expertise, or resources over that required to create an allele that still contains a selectable marker. The paradigm has obvious utility in the production of subtle and conditional mutations that require generation of alleles with minimal structural alterations. Because the presence and transcriptional activity of selectable markers can contribute to phenotypes in an unanticipated and unwanted manner ...the approach will also [be] useful for generating null alleles.

(Specification page 15, bottom to page 16, line 12). Applicants have shown that loxP-flanked targets remain and are not recombined to a functionally significant extent in the somatic tissues of

mice that contain ProCre transgenes, but that more than 90% of the progeny sired by these males inherit a Cre-recombined target (Specification, page 23, lines 17-22). Such embryonic stem cells can be crossed with a wild-type genome of the same genus to obtain offspring in which the transcriptionally active marker gene has been excised and a new allele is created.

Despite the Examiner's fear that a lethal allele could not be created without destroying the embryo, Applicants teach that the allele can be a lethal allele because "germline transmission would be restricted to rare chimeras in which the level of chimerism was low enough in tissues affected by the mutation to allow survival and high enough in the germline to allow transmission, especially if the Cre transgenes are expressed during early embryogenesis from the human cytomegalovirus (CMV) minimal promoter ..., the andenovirus EIIa promoter ..., or the zP3 promoter" (Specification, page 18, lines 25-30). Further, lethality of the allele can be masked by cross-breeding with a wild-type partner in the invention methods.

In addition, Applicants submit that it is standard practice in the art to illustrate the function of a DNA construct, and thereby enable it under 35 U.S.C. § 112, First Paragraph, by using the construct to express a marker gene as a surrogate for a functional protein. More specifically, those of skill in the art will appreciate that the expression of functional genes under the control of a tissue specific promoter was well known in the art at the time the present application was filed. For example, FLP expression under the control of a tissue specific promoter is well accepted in the art. The Examiner has provided no reason to question the operability of this art recognized system in the present invention.

Further, the Examiner has provided no reasons to support the conclusion that expression of a marker gene by a genetic expression construct fails to provide adequate enablement for the same construct to express a functional gene product in the place of the marker gene in a living system other than a mouse. It is well settled law that "the PTO has the burden of giving reasons, supported by the record as a whole, why the specification is not enabling. . . . Showing that the

disclosure entails undue experimentation is part of the PTO's initial burden (***In re Angstadt***, 190 *USPQ* 214, 219 (C.C.P.A. 1976). A corollary pertaining to enablement issues holds that the Specification is deemed to be supplemented by all the teachings of the art at the time of its filing. For example, in *Lindemann Maschinenfabrik GmbH v. American Hoist & Derrick Co.*, the court held that "[t]he question is whether the disclosure is sufficient to enable those skilled in the art to practice the claimed invention, hence the specification need not disclose what is well known in the art" (221 *USPQ* 481, 489 (Fed. Cir. 1984)).

In the rejection for lack of enablement herein, the Examiner fails to apply these legal principles. On the one hand, the Examiner admits that "the techniques used to create transgenic mice and rats are similar" and cites an art recognized treatise (Robl and Heideman) concerning the superovulation of rats that was published before the filing date of the present application. Yet, with regard to the enablement of claims 12-16 and 18-44, the Examiner alleges that the disclosure is enabling only with respect to the production of stem cells from mice because "the specification does not teach how to superovulate female rats" (Office Action, page 10). Clearly, if the techniques for creating superovulation of rats are known, the teachings of the Specification are to be considered as supplemented by this kind of teaching as well as that pertaining to other types of animals more commonly grouped together under the rubric of "livestock."

Thus, Applicants respectfully submit that the rejection for lack of enablement of the full scope of the invention is flawed in failing to provide adequate reasons in support of the allegations upon which the rejection is based and for requiring the Applicants to provide disclosure in support of the invention regarding aspects of its performance that were well known in the art at the filing date of the application. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection Under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully traverse the rejection of claims 2, 22 and 24 for alleged indefiniteness under 35 U.S.C. § 112, Second Paragraph. Specifically with respect to claim 33, the Examiner has alleged the indefiniteness of the term "essential" because of an alleged lack of definition of this term in the specification and in the art. However, it is respectfully submitted that the phrase "an essential portion of a gene of interest" as used in claim 33 is not indefinite because those of skill in the art would understand that an "essential" portion of DNA is a portion of the sequence that is required to produce a biological function of the DNA in the cell, whether the DNA encodes a functional protein or a transcription regulatory region, or whether the DNA is part of an intron whose function cannot be determined. Stated another way, those of skill in the art would understand that deletion of a portion of DNA that is not "essential" does not interfere with the native biological function of the DNA, or its protein product in the cell or organism.

With respect to claim 40, the Examiner alleges that the term "conditional promoter" is indefinite due to an alleged lack of definition of the term in the Specification and existence of a variety of meanings in the art. Further, the Examiner states:

> It is unclear whether the applicants consider MP1 promoter a conditional promoter because it promotes expression of gene only in sperm tissue conditions or whether the applicants intend to claim some other condition required for expression.

(Office Action, page 12). Contrary to the Examiner's assertion, it is respectfully submitted that the Specification provides a clear definition for the term "conditional promoter," which definition states:

> Conditional promoters contemplated for use in practice of the present invention comprise transcription regulatory regions that function maximally to promote transcription of mRNA under inducing conditions

(Specification, page 7, lines 16-19). To illustrate the meaning of the term "conditional promoter," Applicants list a number of examples of inducible promoters suitable for use in the practice of the invention (Specification, page 7, lines 21-19). Thus, it is submitted that the Applicants have provided a clear definition for the term at issue. However, in the interest of expediting prosecution of this application, claim 10, as well as claims 15, 31, 36, 39, 41 and 42, are amended herein to replace "conditional promoter" by the term "inducible promoter," thereby rendering moot the rejection of claim 40.

With regard to claim 13, Applicants respectfully disagree with the Examiner's assertion that the phrase "recombination target sites" is indefinite based on an alleged lack of definition in the Specification and uncertainty as to whether the Applicants intend the term to include endonuclease cut sites as recombination target sites (Office Action, page 12). Applicants submit that the terms "recombination site" and "recombination target sites" would be definite to those of skill in the art in the context of the teaching of the specification and the subject matter of the claims in which such terms are used. Nevertheless, to expedite prosecution and to further clarify the meaning of these terms, claims 13, 15, 25, 30 and 31 are amended herein to substitute "recombinase recombination site(s)" and "recombinase recombination target site(s)" for the phrases "recombination site(s)" and "recombination target site(s)", respectively. It is submitted that these amendments clarify that the recombination sites are those specific to recombinase enzymes and that amended claims 13, 15 25 30 and 31 are definite under 35 U.S.C. §112, Second Paragraph.

With regard to claim 44, Applicants respectfully disagree with the Examiner's assertion that the term "pluripotential" is indefinite because of an alleged lack of clear understanding of its meaning in the art and because "[i]t is not clear whether the applicants consider pluripotent ES cell[s] to have two potential routes of differentiation or twenty potential routs [sic] of differentiation" (Office Action, page 13). Contrary to the Examiner's assertion, Applicants respectfully submit that the *Concise Dictionary of Biomedicine and Molecular Biology* provides

a clear definition of 'pluripotent stem cell' as "cells capable of differentiating into different cell types" (Ed. Pei-Show Juo, CRC Press, Boca Raton, 1996, page 738). This authority gives no indication that the number of cell types into which a pluripotent stem cell can divide is arbitrarily limited to two or any other number. Further, it is well known in the art that the type(s) of cell into which a pluripotent stem cell can differentiate is determined by the biological context in which it is found. In view of the Examiner's failure to provide any reason in support of the allegation that the term is indefinite in the art, Applicants respectfully submit, in reliance upon the above definition, that it is not. Accordingly, the term "pluripotential" has been maintained in claim 44.

Also with regard to claim 44, Applicants respectfully disagree with the Examiner's assertion that the term "livestock" is not defined in the specification and that it is unclear whether Applicants consider mice livestock (Office Action, page 13). Applicants respectfully submit that although the term "livestock" *per se* is not defined in the Specification, a clear definition of the scope of animal species into which the invention constructs can be introduced to practice the invention methods is provided in the Specification, as follows:

> . . . the above-described constructs can be introduced into a variety of animal species, such as, for example, mouse, rat, rabbits, swine, ruminants (sheep, goats and cattle), . . . poultry, fish and the like.

(Specification, page 8, lines 31-34). This list of animal species is consonant with the definition of 'livestock' in *Webster's II New College Dictionary*: "Domestic animals, as cattle or horses, raised for home use or profit" (Houghton Mifflin Company, Boston, 1995, page 641). Applicants submit that those of skill in the art would understand that "livestock" includes mice (which are often raised *en masse* for profit as laboratory animals) as well as other animal species of the type listed in the Specification. Accordingly, Applicants' submit that use of the term "livestock" in claim 44 comports with the ordinary dictionary definition of the word and, therefore, is to be considered definite to those of skill in the art.

Based upon the amendments and the above remarks, Applicants submit that the subject matter of all pending claims is definite under 35 U.S.C. § 112, Second Paragraph.

The Rejection Under 35 U.S.C. § 102

Applicants traverse the rejection of claims 1, 2 and 4 as allegedly being anticipated by Lewandoski *et al. (Current Biology* 7:148-151, 1997) (hereinafter "Lewandoski") on the grounds that Applicants reduced the present invention to actual practice in the United States prior to the publication date of the Lewandoski reference. In a fully executed Declaration submitted herewith under the provisions of 35 U.S.C. §131, the co-inventors of the subject matter of the present claims, Stephen O'Gorman and Geoffrey Wahl, assert that the invention was reduced to actual practice in the United States before the publication date of Lewandoski. In support of this assertion, attached to the Declaration are true copies (except for the redaction of dates) of pages of laboratory notebooks from The Salk Institute for Biological Studies in La Jolla, California, that describe eight steps taken by the inventors to reduce the invention to practice. Based on the Declaration of the inventors herein "swearing behind" the publication date of the Lewandoski reference, Applicants submit that Lewandoski is not prior art under 35 U.S.C. § 102 and that the rejection for alleged anticipation is therefore moot.

The Rejection Under 35 U.S.C. § 103

I. Applicants respectfully traverse the rejection of claims 1-2, 4-5, 10-16 and 18-19 and 24-44 for alleged obviousness over Gu et al. (Science :265-1-3-106, 1994, hereinafter "Gu") in view of Zambrowicz *et al.* (*Biology of Reproduction 50*:65-72, 1994, hereinafter "Zambrowicz"), and Lakso et al. (Proc. Natl. Acad. Sci, 93:5860-5865, 1996). Applicants respectfully disagree with the characterization of the combination of the references in the Office Action, which states:

> [I]t would have been obvious to replace the T-cell specific promoter used in the Cre-loxP system taught by Gu et al. with the MP1 promoter taught [by] Zambrowicz et al. to obtain sperm-specific expression of Cre recombinase. One of skill would have been motivated

> to combine the Cre-loxP system and the MP1 promoter to eliminate in vitro ES cell manipulation to obtain germ-line transmission of a recombined transgene. Motivation to combine is provided by Lakso et al. who state the need to obviate the extended culture of ES cells in order to expose the ES cells to recombination by directing recombination to the embryo. As a sperm-specific promoter operatively linked to Cre recombinase would produce sperm in which recombination occurs then used [sic] to fertilize eggs and obtain recombined embryos, thus obviating the extended culture and genetic manipulation of ES cells to obtain the same thing.

(Office Action, page 15).

Applicants' claims distinguish over the references relied upon, taken alone or in combination, by requiring nucleic acid constructs comprising a germline-specific, tissue specific or inducible promoter operatively associated with a recombinase coding sequence (claims 1, 2, 4-6 and 8-15) and embryonic stem cells containing such a nucleic acid construct. The embryonic stem cells can optionally further contain one or more of (1) a nucleic acid fragment flanked by two recombination target sites that are different than the recombination target sites which flank the selectable marker (2) a nucleic acid construct comprising a conditional promoter operatively associated with a recombinase coding sequence, or (3) a nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence. Methods are provided for using the embryonic stem (ES) cells so modified to produce a recombinant allele in a transgenic animal by introducing a nucleic acid fragment flanked by at least two recombinase recombination sites into the modified ES cells and passaging the genome derived from the ES cells through gametogenesis. Development of the ES cells through gametogenesis and crossing of the transgeneic gamete with a wild type gamete results in a germ-line from which first generation transgenic animals can be obtained having an allele that results from deletion of the polynucleotide target.

In addition, claims 28-31 provide a method for excision of the transcriptionally active selectable marker from embryonic stem cells containing nucleic acid constructs having a germ-line specific promoter operatively associated with a recombinase coding sequence, a

transcriptionally active marker gene under the control of a germ-line specific promoter flanked by recombinase recombination sites, and a polynucleotide flanked by recombinase recombination sites. Such ES cells can be isolated using the marker, and the marker gene and its transcriptional sequences can be excised from the recombinant ES cells before using the ES cells to create a transgenic offspring, thereby assuring that any phenotype detected in the offspring can be confidently ascribed to the presence of the allele and not to an interfering effect resulting from the gene encoding the marker.

The combined disclosures of the references cited fail to suggest Applicants' claimed constructs and methods of use. In particular, Applicants respectfully disagree with the statement in the Office Action that:

> it would have been obvious to replace the T-cell specific promoter used in the Cre-loxP system taught by Gu et al. with the Mp1 promoter taught [by] Zambrowicz et al. to obtain sperm-specific expression of Cre recombinase.

(Office Action, page 15).

First, as the Examiner admits "Gu et al. do not teach the production of a nucleic acid construct comprising a germline-specific promoter or ES cells comprising a nucleic acid construct comprising a germline-specific promoter operatively linked to a Cre recombinase gene" (Office Action, page 14). Applicants respectfully submit that the Office Action ignores the many additional deficiencies of Gu for disclosing or suggesting the constructs and methods of the present claims.

For example, Gu discloses that a two step process, involving two separate cloning steps, is required to achieve a transgenic ES cell carrying a desired allele or a transgenic animal having a desired allele (Gu, Figures 1A and B and description, page 104). In the first step, DNA is used containing three loxP sites, two of which flank the selection marker genes for neomycin resistance (Neo) and HSV-tk, while the second and third flank regions of a germ line target gene

(polβ). Cells transformed with such DNA constructs in which homologous recombination takes place are selected. The neo gene is deleted in an *in vitro* step. Then, in the second step, such ES clones are transiently transfected with plasmid encoding Cre under control of a T-cell specific promoter so that a recombination event occurs only once in some of the transfected ES cells. Thus, in Gu's method, a two step process is required to arrive at ES cells from which the marker gene has been removed. With regard to production of transgenic animals using the loxP homologous recombination procedure disclosed by Gu, two transgenic animals are required to obtain offspring bearing the desired mutation in the germ line (Gu, page 105, third column).

Thus, in addition to being silent regarding use of a germ-line specific promoter operatively associated with a recombinase gene so that the recombinase gene is expressed only in germ-line tissue (as admitted by the Examiner), Gu fails to suggest substitution of a germline specific promoter for a T-cell promoter to arrive at a single cloning step procedure for obtaining ES cells from which transgenic animals can be obtained by passaging the ES cells through gametogenesis and breeding with a wild type animal.

Zambrowicz does not overcome all of these deficiencies in the disclosure of the primary reference. Zambrowicz discloses that testis nuclear proteins bind only to a specific region of the testis-specific Prm-1 promoter so as to regulate spermatid-specific transcription and, more specifically, "so as to recognize sequences within and immediately adjacent to a CRE-like sequence" (page 70, Col. 1 bottom, to Col. 2 top). However, Zambrowicz discovered that there are three other regions (C, B, and O) within the same promoter that are bound by nuclear proteins that are not testis-specific (See Discussion, page 70). Thus, Zambrowicz discloses the binding of testis-specific proteins to a "Cre-like" region, but suggests as well that other "non-testis-specific" proteins will also bind to the Prm-1 promoter. Further, Zambrowicz discloses that the testis-specific proteins appear after Day 12 of spermatogenesis (see Abstract).

In addition, Zambrowicz is silent regarding methods for using the combination of any germ-line promoter in a nucleic acid construct containing a gene that encodes Cre, or any other germ-line promoter in a nucleic acid construct, to obtain insertion or deletion of a target gene into embryonic stem cells. Moreover, Zambrowicz, like Gu, is silent regarding use of a germ-line specific promoter operatively associated with a recombinase gene so that the recombinase gene is expressed only in germ-line tissue. In addition, Zambrowicz fails to suggest that a marker gene can be deleted by passage of embryonic stem cells through gametogenesis, thus allowing production of transgenic animals from such embryonic stem cells wherein the transgenic marker has been deleted and a new allele is accomplished in a tissue specific or in an inducible manner, as in Applicants' claims. Thus, Applicants respectfully submit that Zambrowicz does not cure the deficiencies of Gu as described above.

Applicants further respectfully disagree with the assertion in the Office Action that Lakso provides motivation for combining the disclosures of Gu and Zambrowicz to arrive at the subject matter of Applicants' claims 1-2, 4-5, 10-16 and 18-19 and 24-44. Regarding the alleged motivation provided by Lakso to combine the Cre-loxP system of Gu with the M1 promoter taught by Zambrowicz, Applicants submit that the statement of Lakso cited in the Office Action regarding the desire for a means to excise a selectable gene product to prevent its unwanted effect after it has served its purpose during selection (Lakso, page 5865, Col. 1 first full paragraph) is taken out of context. Lakso's disclosure pertains to the efficiency of Cre-mediated DNA recombination in the specific context of the zygote state of embryo development using the adenovirus EIIa promoter to direct Cre expression to preimplantation embryos (Lakso, page 5860, Col. 1, bottom). In this context, Lakso discloses deletion of the loxP flanked neo gene by crossing of homozygous EIIa-cre mice with homozygous mice carrying a targeted insertion of a single copy loxP-neo-loxP cassette. Regarding the failure in these experiments of a substantial proportion of the F1 mice to undergo Cre-mediated neo excision, Lakso observed:
"...persistence of the undeleted NEO allele in a proportion of cells may reflect Cre action past

the zygote stage, resulting in some mosaicism." (Lakso, page 5861, second Col.). Thus, Lakso's method is admittedly inefficient.

Further, Applicants submit that Lakso does not suggest how to achieve removal of the neo gene other than by interbreeding of a transgenic line carrying loxP-flanked neo with a mouse carrying EIIa-cre to obtain first generation progeny in which the neo sequence was efficiently deleted from all tissues tested (Lakso, Abstract). Lakso is silent regarding how to create a first generation mouse line from which neo has been deleted and in which a recombinase-mediated recombination has occurred.

Thus, the differences between Applicants' constructs and methods and those disclosed by Lakso are important and numerous, and the Examiner has provided no reason in support of the allegation that Lakso's desire for a better way would lead those of skill in the art to turn to the disclosure of Gu and Zambrowicz to arrive at Applicants' nucleic acid constructs, ES cells containing them, and methods of using such constructs and ES cells to obtain transgenic animals, as required by claims 1-2, 4-5, 10-16 and 18-19 and 24-44. Accordingly, Applicants respectfully submit that Lakso fails to motivate those of skill in the art to combine the teachings of Gu and Zambrowicz and further that, in fact, the prior art cited by the Examiner is being read in light of the teaching of the present specification. It is well settled in patent law that the Examiner is not allowed to selectively pick and choose elements or concepts from the various references so as to arrive at the claimed invention using the claims as a guide. Hindsight is not a proper criteria for resolving the issue of obviousness.

Based on the foregoing analysis of the references cited, Applicants submit that the rejection for alleged obviousness is based upon hindsight reconstruction of the disclosure of the references in light of Applicants teachings, and that *prima facie* obviousness of claims 1-2, 4-5, 10-16, 18-19 and 24-44 is not established by the Gu-Zambrowicz-Lakso combination of references.

II. Applicants respectfully traverse the rejection of claims 1, 6-9 and 20-23 for alleged obviousness over Gu in view of Zambrowicz and Lakso as applied in Section I above and further in view of Onouchi *et al.* (*Mol. Gen. Genet* 247:653-660, 1995, hereinafter "Onouchi"). Applicants respectfully disagree with the characterization of the combination of references in the Office Action, which states:

> The combined teachings of Gu et al., Zambrowicz et al. and Lakso et al. do not teach the production of a nucleic acid construct comprising FLP recombinase or the R gene product of *Zygosaccharomyces*.
>
> However, . . . it would have been obvious to replace the Cre recombinase gene with the FLP recombinase gene or the R gene product of *Zygosaccharomyces* taught by Onouchi et al. Motivation is provided by Onouchi et al. by stating the Cre-lox, FLP-FRT and R-RS system are all similar and cause recombination . . . One of skill would have had a reasonable expectation of success using the FLP-FRT or R-RS system and the MP1 promoter to obtain expression of a marker gene.

(Office Action, page 16). Applicants repeat here with respect to claims 1, 6-9 and 20-23 the arguments above concerning the insufficiency of the Gu-Zambrowicz-Lakso combination of references for disclosing or suggesting the invention nucleic acid constructs and their use in methods of making transgenic stem cells and/or transgenic animals derived from such stem cells.

With regard to the assertion in the Office Action that Onouchi motivates substitution of the FLP-FRT or R-RS system and the MP1 promoter to obtain expression of a marker gene, Applicants respectfully submit that the disclosure of Onouchi does not overcome the deficiencies of the Gu-Zambrowicz-Lakso combination of references discussed above. For instance, Onouchi does not disclose ES cells containing a nucleic acid construct comprising an FLP-FRT or R-RS system, or a method of excising a marker gene from the germ line of an ES cell having a nucleic acid construct with a target sequence flanked by recombinase specific recombination sites integrated into the germ line. Nor does Onouchi disclose the obtaining of a first generation transgenic animal from such an ES cell by crossing the genome of the ES cell with a wild type genome.

Accordingly, Applicants respectfully submit that the combined disclosures of Onouchi, Gu, Zambrowicz, and Lakso fail to teach or suggest the subject matter of present claims 1, 6, 8, 9, and 20-23.

Conclusion

Applicants respectfully request reconsideration and withdrawal of the objection to the specification and rejections of the claims, and passage of the claims to allowance. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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